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Differential detectability of polymorphic warning signals under varying light environments

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ABSTRACT

The striking colour-pattern variation of some aposematic species is paradoxical because selection by predators is expected to favour signal uniformity. Although the mechanisms allowing for the maintenance of such variation are not well understood, possible explanations include both non-adaptive processes like drift and gene flow; and adaptive processes, such as an interaction between natural and sexual selection, spatial and temporal variation in selection, a link between behaviour or other fitness-related traits and phenotype, and predators' ability to generalise among different signals. Here we test whether warning-signal polymorphisms, such as that of dyeing poison frogs (*Dendrobates tinctorius*), could be maintained by differences in detectability among morphs. We did experiments in the wild using wax models with different aposematic colour patterns vs. cryptic ones, and examined the attack rates by wild predators over time. We also tested the detectability of different aposematic morphs by 'human predators' under different light environments. We found that cryptic frog models were attacked more than aposematic models, but there were no differences in bird attack rates towards the different aposematic morphs. However, we found that detectability of different morphs depends both on predator experience and light environment. We suggest that the interaction between differential detectability and signal efficiency among morphs in different light conditions could be a mechanism aiding to the maintenance of warning-signal polymorphisms. Our results highlight the importance of considering the light environment at which predators have their first encounters with aposematic prey for future studies on predation in the wild.

1. Introduction

The existence of intra-populational colour-pattern variation in aposematic species is paradoxical because the stabilising selection exerted by predators is expected to favour signal uniformity (Endler, 1988; Endler and Mappes, 2004; Joron and Mallet, 1998; Mallet and Joron, 1999; Rojas and Endler, 2013; Rowland et al., 2007). Despite this paradox, several aposematic species exhibit variable colour patterns along their distribution range (polytypism; e.g. poison frogs (Brusa et al., 2013; Myers and Daly, 1976, 1983)) or within populations (polymorphisms; lady birds, butterflies, moths, poison frogs; O'Donald and Majerus, 1984; Ueno et al., 1998; Mallet and Joron, 1999; Brakefield, 1985; Rojas and Endler, 2013; Nokelainen et al., 2014), but the mechanisms behind the maintenance of this variation are still unclear, especially at the intra-populational level.

Recent approaches suggest that variation in aposematic colour patterns may be maintained by an interaction between natural and sexual selection (Maan and Cummings, 2009; Crothers and Cummings, 2013; Cummings and Crothers, 2013), whereby individuals of certain morphs are, for example, more successful at deterring predators, whereas individuals of other morphs are more successful at attracting mates (Nokelainen et al., 2012). There are many non-mutually exclusive alternative explanations, some of which have recently attracted much attention: (1) a link between behaviour or other fitness-related traits and phenotype, for example through morph-specific investment in immune defences across variable environments (Nokelainen et al., 2013), or through specific signal-behaviour associations (Rojas et al., 2014); (2) spatio-temporal variation in selection, for example in predator community composition (Nokelainen et al., 2014), by generating a selection mosaic throughout the prey geographic range; (3) hybridisation among geographic variants (Medina et al., 2013); and (4) predator generalisation or relaxed selection on a warning signal

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(Amézquita et al., 2013; Richards-Zawacki et al., 2013), which occurs when predators learn to avoid a signal with salient cues such as specific colours or patterns (or both), and then expand this aversion to other signals provided that they are similar enough to the one learnt in the first place (Darst and Cummings, 2006; Svádová et al., 2009; Ham et al., 2006). In addition to possible opposing selection processes, almost certainly the additional influence of frequency dependent selection or gene flow among populations is needed. Here we explore one more possible explanation that would allow for the co-existence of polymorphic warning signals: differences in detectability between (or among) the morphs.

Signal detectability can be exacerbated or hindered in particular habitats that differ, for example, in their light environment (Endler, 1993). Differences in light environment might render differences in conspicuousness and, at the same time, in the efficiency of communication which may in turn have direct effects on fitness (Endler, 1993, 1986). For animals inhabiting forests this is particularly important because of the combination of forest geometry and the different spectral environments available both at fine spatial scales and throughout the day (Endler, 1993). Light conditions may affect fitness directly because changes in the light environment may turn inconspicuous colour patterns into conspicuous and vice versa, influencing communication efficiency (Endler, 1993, 1991). Therefore, the colour patterns and behaviour of each species are expected to maximise its visual contrast during signal display (Endler and Théry, 1996). This has been shown in the context of sexual displays (Endler and Théry, 1996; Heindl and Winkler, 2003; Théry and Endler, 2001), but may also affect predator–prey interactions. If some signals were not only easier to learn, but also easier to detect by naïve predators than others, lower detectability could in contrast convey extra protection to defended individuals bearing signals that are more difficult to learn, or that are less memorable, aiding to the maintenance of colour pattern polymorphism.

One of the biggest difficulties in the study of predator–prey interactions is that predation events are rarely observed in nature. Witnessing predation on species with protective colouration is especially challenging because they can be either particularly hard to detect by predators, such as those species whose protection relies on camouflage, or because predators have learned to avoid them to some extent, such as warningly coloured, defended species. Most of the information regarding the role of selective pressures, such as predation, in the evolution of animal colour patterns comes from experiments in semi-natural conditions (Chai, 1986, 1988; Langham, 2004; Thomas et al., 2004; Allen and Clarke, 1968), or in controlled setups in the laboratory, with either live (Exnerová et al., 2007, 2006; Svádová et al., 2009; Gamberale-Stille and Tullberg, 1999; Nokelainen et al., 2012; Forsman and Appelqvist, 1998; Marples et al., 1994) or model (Alatalo and Mappes, 1996; Ham et al., 2006; Ihalainen et al., 2008; Lindström et al., 2001, 1999a,b, 2006; Bond and Kamil, 1998, 2002, 2006) prey subject to visual predators (e.g. birds). Humans have also been used as 'predators' in order to test for the protective nature of different colour patterns or colouration traits on photographs or virtual prey (Summers and Clough, 2001; Hagman and Forsman, 2003; Sherratt et al., 2004; Stevens et al., 2008; Beatty et al., 2005; McGuire et al., 2006; Fraser et al., 2007; Karpestam et al., 2012; Bohlin et al., 2012), but only rarely (Karpestam et al., 2013) in the natural environment of prey. Interestingly, some of these experiments have indicated a strong resemblance between the perceptual abilities of birds and those of humans under laboratory conditions (Penney et al., 2012) and a correspondence between human detection of different colour patterns and their relative survival in the wild (Karpestam et al., 2013).

Several studies have used either plasticine or paraffin wax models in order to quantify predation attempts on individuals bearing different colour patterns in the wild (e.g. Pfennig et al., 2007; Brodie, 1993; Nokelainen et al., 2014; Amézquita et al., 2013). However, these studies have four main limitations: (1) Lack of movement: although aposematic organisms such as some lepidopteran larvae are not particularly agile and remain stationary for long periods of time (Bowers, 1993), many aposematic species tend to be active foragers that wander around freely, and are therefore easily detected by motion-oriented predators like birds. The use of stationary models might therefore lead to an underestimation of predation attempts. (2) Lack of first hand information about predator identity and their behaviour is missing and, therefore, it is impossible to know if a certain model survives because its signal is efficient or because it is simply harder to find. (3) Unnatural odour and not necessarily very natural colouration. (4) Lack of secondary defence: clay/wax models do not have the chemical or physical defences that make the model species truly aposematic. Therefore, in such models, the aposematic signal is incomplete. Although the clay or wax models do not have any reward either, there is a possibility that the lack of punishment overestimates the attack risk of aposematic prey if the same predators are responsible for more than one attack in such studies. Some of these limitations have been addressed lately. For example, a recent study reported an increased number of predator attacks in moving vs. stationary models, both cryptic and aposematic (Paluh et al., 2014); two other studies controlled the relative conspicuousness of different morphs by placing the models on standardised backgrounds (Hegna et al., 2011; Nokelainen et al., 2014), and a fourth one provided measurements of the reflectance of both the real animals and the models to ensure that their colouration did not differ significantly (Valkonen et al., 2014). However, experiments with defended models, to our knowledge, have not yet been done.

The dyeing poison frog, *Dendrobates tinctorius*, is a species with potent skin alkaloids (Daly et al., 1987) and enormous inter- and intra-populational variation in colour patterns (Fig. 1 A–D; Wollenberg et al., 2008; Noonan and Gaucher, 2006; Rojas and Endler, 2013), known to invade canopy gaps as soon as they form (Rojas, 2012; Born et al., 2010). Individuals with simpler colour patterns (*sensu* Endler, 2012) are the first to invade these environments as soon as they become available (i.e. within hours; Rojas and Endler, unpublished data). Individuals with complex patterns, on the other hand, arrive later (Rojas and Endler, unpublished data). According to signal detection theory, simple signals are more detectable and easier to remember (Macmillan and Creelman, 1991). Thus, from the prey point of view, this raises the question whether individuals with simpler colour patterns bear a more efficient warning signal and are, therefore, better at training predators. If so, this would explain why individuals with simpler colour patterns would venture to invade new environments rapidly. From the predator point of view, the question is not only what colour patterns are more efficient as warning signals, but also whether certain colour patterns are more easily detected under a particular light environment (i.e. a treefall gap). Bird species exposed to a new gap might encounter prey that they have not encountered before, and seeing such prey for the first time under the light conditions of a gap may affect their encounter rates in a darker environment (i.e. closed forest) and vice versa, simply because the same colour patterns might not look the same in both environments. Here we use two different experiments (one with wax models and natural predators, and another one with wax models and human predators) in order to determine whether differential detectability in varying light environments could aid in the maintenance of warning signal variability. More specifically, we aimed to test the following hypotheses: (1) individuals with simpler colour patterns are less attacked over time (they are learnt more effectively by predators); (2) individuals with complex colour patterns, if less efficient, can afford to exist because they are less detectable; and (3) predators can detect simple colour patterns more easily in the light conditions provided by a canopy gap.

2. Methods

2.1. Study site and species

The dyeing poison frog *Dendrobates tinctorius* is one of the largest species within the family of poison frogs (Dendrobatidae), with adult sizes that range between 35 and 50 mm (Rojas and Endler, 2013). This species inhabits the Easter Guiana shield, especially next to canopy gaps, at elevations between 0 and 600 m (Noonan and Gaucher, 2006). Their major predators remain unknown, although the results of previous experiments with clay models in the wild and some anecdotal observations on other species of poison frogs point mainly at birds (Comeault and Noonan, 2011; Noonan and Comeault, 2009).

D. tinctorius exhibits bright colouration, which varies both within- (Rojas and Endler, 2013) and between- (Wollenberg et al., 2008; Noonan and Gaucher, 2006) populations, and has skin alkaloids that are thought to be predator-deterrent, which allow for its classification as *aposematic* (Summers and Clough, 2001). At the study population its colouration is extremely variable (Fig. 1A–D), with all individuals sharing a black dorsal background that can be almost covered with yellow or exhibit just a few yellow lines or spots, and most exhibiting blue legs (Rojas and Endler, 2013). The variation is therefore continuous, rather than discrete in the classical sense of a colour polymorphism (Ford, 1945). However, this variation can be grouped roughly into four categories (Fig. 1A–D; B. Rojas pers. observ. of over 700 frogs identified individually at the study site): (1) 'yellow', where most of the dorsum is covered by this colour, with a black spot of varying size within the yellow area (Fig. 1A); (2) 'double ring', where the yellow markings form an eight-shaped pattern (Fig. 1B); (3) 'spotted', characterised by yellow spots in the head area, and interrupted, irregular yellow markings in the dorsal region (Fig. 1C); and (4) 'ring', which exhibits a thin, often uninterrupted, yellow oval (Fig. 1D). A previous study reported a thorough analysis of colour patterns in this species (Rojas and Endler, 2013) by quantifying several variables of dorsal colour pattern geometry such as pattern simplicity, pattern elongation and proportion of dorsal yellow. Such quantification is

Fig. 1. (A–D) Natural colour patterns in a population of the frog *Dendrobates tinctorius*; (E–F) aposematic morphs used for Experiment 1 with wild predators (E: Yellow, F: Double ring). In addition to E–F, morphs G–H (G: Spotted, and H: Ring) were used as well for Experiment 2, with human predators. Examples of attacks by birds (I–J), insects (probably roaches; K) and mammals (L) during Experiment 1. See Methods section for details.

based on a method (Endler, 2012) that takes into account the number of transitions between adjacent colours (in this case, between black and yellow). Simpler patterns have a lower number of transitions between adjacent colours than more complex ones, both in the vertical and the horizontal axes (see Endler, 2012 for details on calculations). According to these measurements, 'yellow' individuals tend to have simpler patterns, followed by 'ring' and 'spotted' individuals, whereas frogs with a 'double ring' tend to have the most complex ones (Fig. S1). Pattern simplicity is not correlated with the amount of dorsal yellow in this species (Rojas and Endler, 2013).

This study was done between January and February 2013, during the breeding season of the species, at Camp Pararé, Les Nouragues Research Station, French Guiana (3°59′N, 52°35′W). Although within the same research station where *D. tinctorius* has been previously studied, at the site(s) chosen for both experiments described below, *D. tinctorius* does not occur.

2.2. Experiment 1. Predation attempts on wax models of different colour patterns

Dendrobates tinctorius has been documented to invade tree-fall gaps within hours and days of their formation (Rojas, 2012; Born et al., 2010). Individuals that arrive first to these 'novel' habitats are individuals with simpler colour patterns (*sensu* Endler 2012; Rojas and Endler, unpublished data; see above). That would make sense if simpler patterns were more efficient warning signals, which would place the individuals bearing them in advantage when invading a novel environment. We placed 900 paraffin wax models, 300 of a 'simple' colour morph (yellow; Fig. 1A), 300 of a 'complex' colour morph (double ring; Fig. 1B), and 300 brown (not warningly coloured), distributed in equal numbers in 15 spots that resembled a canopy gap. These spots were located in a site where individuals of *Dendrobates tinctorius* had not been seen during previous censuses (Devillechabrolle, 2011), i.e., a novel environment. Each of the 15 sites used had an area of approximately 100 m^2, and was separated from the nearest site by a minimum distance of 100 m. Sixty wax frogs (10 brown (defended), 10 brown (non defended), 20 simple, and 20 complex) were haphazardly placed in a set grid, with inter-model distances between 1 and 1.5 m. The model density of each site was therefore 60 frog models per 100 m^2; this density is far beyond the natural density of 4.28 individuals per 100 m^2 observed at a population located approximately 2 km from the site were models were placed (Courtois et al., 2012), but similar to the densities registered immediately after a treefall occurs (over 45 frogs in a similar-sized area at once; BR pers. observ.). We simulated these high densities because we ultimately aimed at understanding the dynamics of the differential gap invasion by individuals with different colour patterns.

We made the models by filling a silicon-rubber template with paraffin wax (Amézquita et al., 2013). All models were painted afterwards with odourless, non-toxic paint according to the colour treatment. Both aposematic morphs were then made unpalatable by spraying them with a solution of 10% chloroquin phosphate (hereon referred to as chloroquin), which has been shown to be distasteful yet harmless for birds in laboratory experiments (Alatalo and Mappes, 1996). Half (150) of the brown models were also made unpalatable following the same procedure in order to distinguish the effect of distastefulness from the effect of colouration. All models were placed the same day and examined after 3, 5, 7, 9 and 11 days in the field in order to check whether there were changes in predation rates on the different morphs over time. On each of these occasions, predation attempts were recorded and the models attacked or missing were replaced. Bird attacks were identifiable by V-shaped and stab-like marks (Fig. 1I, J), as reported in previous studies (Brodie, 1993; Saporito et al., 2007; Noonan and Comeault, 2009; Nokelainen et al., 2014). Although other animals like rodents and insects can 'attack' the models (Comeault and Noonan, 2011; Noonan and Comeault, 2009; Saporito et al., 2007), at the time of data analysis priority was given to bird attacks, as birds are the most likely visual predator of the frogs (Comeault and Noonan, 2011; Noonan and Comeault, 2009; Alvarado et al., 2013; Maan and Cummings 2012; Master, 1999). Differences among the morphs in attack risk over time were analysed with a survival analysis (Cox regression) using the probability of bird attack as a binary response variable, and the colour morph as a covariate.

2.3. Experiment 2. Detectability of different colour patterns by 'human predators' under different light environments

We chose two sites differing in light conditions: one in a canopy gap and another one in the closed forest (Fig. 2). The sites were separated by approximately 100 m. A 6 m × 6 m quadrat was set at both places and 20 frog models, 5 of each of the 4 morphs considered in this experiment (Fig. 1E–H; yellow (E), double ring (F), spotted (G), ring (H)) were placed randomly before each trial. Twenty-five people, 12 women and 13 men between 21 and 64 years old, participated voluntarily in the experiment. Twelve of them were assigned randomly to the canopy gap site for a first trial, whereas the other 13 people were assigned to closed forest. Participants at both sites were asked to find as many frog models as possible during 30 s. One model of each morph was shown to the participants right before the experiment, in order for them to identify them and learn their names. Each trial was filmed, and from them recorded the number of frogs of each morph found, the order in which they were found and the time taken until the first frog was found. To test for the effect of experience on the rate at which frogs were found, after the first trial each participant was taken to the second site (closed forest for those who started at the canopy gap and vice versa), and asked to do the same. Therefore, half of the participants did the experiment in one order (went from closed forest to canopy gap), and the rest of the participants did the experiment in the opposite order (went from canopy gap to closed forest).

Fig. 2. Canopy cover of each of the two sites used for Experiment 2, with human predators. Canopy gap (left) and closed forest (right).

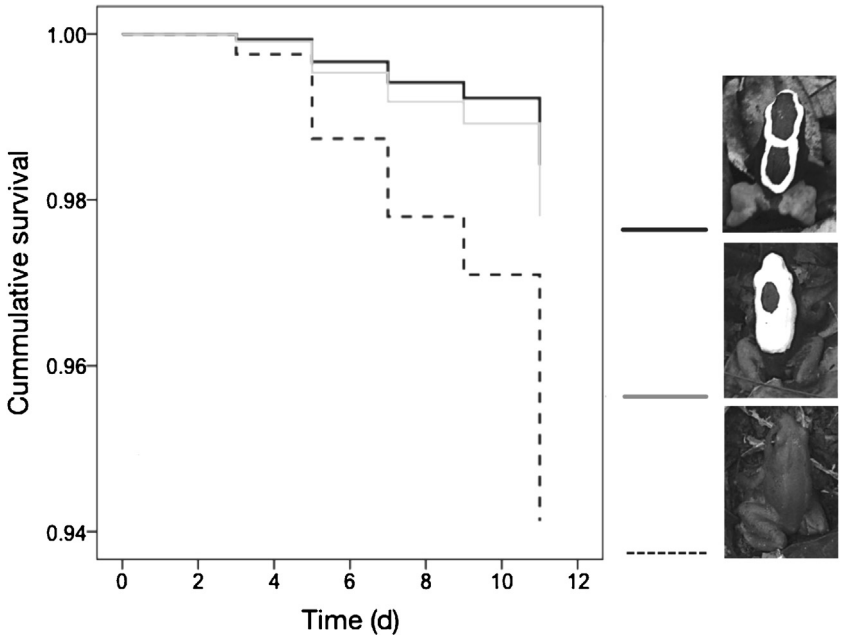

Fig. 3. Cumulative probability of survival to wild predators over time of models with aposematic colouration exhibiting simple (grey) or complex (black) patterns, in comparison to models with cryptic colouration (dashed line).

One participant was excluded from the analysis because of evident problems with his vision, whereas two other participants (one in each order treatment) where excluded because they completed only one trial (i.e., found models in just one light environment). Thus, final analyses were done with the remaining 22 participants. Trials in both sites were run by two of us simultaneously, so that the same light and climate conditions applied. We ran a Generalised Linear Mixed Model (GLMM) with number of models found as a response variable, and the main effects of morph, order and site, and all the possible two- and three-way interactions as explanatory variables. Participant ID was included in the model as a random factor, and the best model was chosen on the basis of differences in AIC.

3. Results

3.1. Experiment 1. Predation attempts on wax models of different colour patterns

We registered 115 attacks (out of 1031 models), 62 of which were attributable to birds (brown = 38, simple = 14, and complex = 10; Fig. 1J,K). The rest of the attacks were attributed to mammals, insects (mostly roaches) or classified as unknown. Only 16 models (11 brown, 2 simple, 3 complex) were missing during the whole study. Because their disappearance could not be attributed to predation, these models were not included in the analyses.

Both aposematic models had a significantly higher survival than brown models over time (Fig. 3; Cox Regression: Wald test = 19.93, df = 2, $P < 0.001$; Simple vs. Control: $z = -3.210$, $P = 0.001$; Complex vs. Control: $z = -3.774$, $P < 0.001$), but we found no differences between the aposematic morphs in the frequency of predation attempts (Fig. 3; Simple vs. Complex: $z = -0.186$, $P = 0.410$). The number of attacks on brown models sprayed with chloroquine ($N = 16$) did not differ significantly from that on undefended brown models ($N = 22$; $\chi^2 = 2.675$, df = 4, two-tailed $P = 0.614$). When non-bird attacks and missing models were included in the analyses, the results did not change (Cox Regression: Wald test = 45.70, df = 2, $P < 0.001$; Simple vs. Control: $z = -5.260$, $P < 0.001$; Complex vs. Control: $z = -5.346$, $P < 0.001$; Simple vs. Complex: $z = 0.140$, $P = 0.889$).

3.2. Experiment 2. Detectability of different colour patterns by 'human predators' under different light environments

Overall, more models were found in the gap (Mean ± SD = 11.38 ± 2.52) than in the closed forest (Mean ± SD = 8.36 ± 2.14; Table 1). This means that light environment influences the detectability of the frogs, making them easier to find in gaps than in the closed forest. We found a marginally significant three-way interaction between morph, site and order (Table 2), suggesting

Table 1
Generalised Linear Mixed Model explaining the total number of wax models found. Participant ID was included as a random factor. The intercept includes Site: Closed forest and Order: from closed forest to gap.

Source	Estimate	Std Error	*z*-value	Pr(>|*z*|)
(Intercept)	2.13553	0.09535	22.398	<0.001
Order1: gap-cf	0.12053	0.13644	0.883	0.377
Site1: gap	0.36211	0.12418	2.916	**0.003***
Order1:Site1	−0.27114	0.18341	−1.478	0.139

Table 2
Generalised Linear Mixed Model explaining the number of models of each morph found. Participant ID was included as a random factor. The intercept includes Morph: Double Ring, Site: Gap and Order: from closed forest to gap.

Source	Estimate	StdError	*z*-value	Pr(>|*z*|)
(Intercept)	−0.08155	0.29892	−0.273	0.7850
Order1	0.01364	0.40437	0.034	0.9731
Site1	−0.68839	0.41733	−1.650	0.0990
morphr	0.41189	0.40506	1.017	0.3092
morphs	0.24583	0.40305	0.610	0.5419
morphy	0.16372	0.40256	0.407	0.6842
Order1:Site1	0.62027	0.55574	1.116	0.2644
Order1:morphr	0.77364	0.56675	1.365	0.1722
Order1:morphs	0.30641	0.54841	0.559	0.5764
Order1:morphy	0.38852	0.54805	0.709	0.4784
Site1:morphr	0.60497	0.58247	1.039	0.2990
Site1:morphs	0.77102	0.58110	1.327	0.1846
Site1:morphy	0.52466	0.57984	0.905	0.3656
Order1:Site1:morphr	−1.92776	0.79515	−2.424	**0.0153***
Order1:Site1:morphs	−1.32326	0.78160	−1.693	0.0905
Order1:Site1:morphy	−1.72393	0.78725	−2.190	**0.0285***

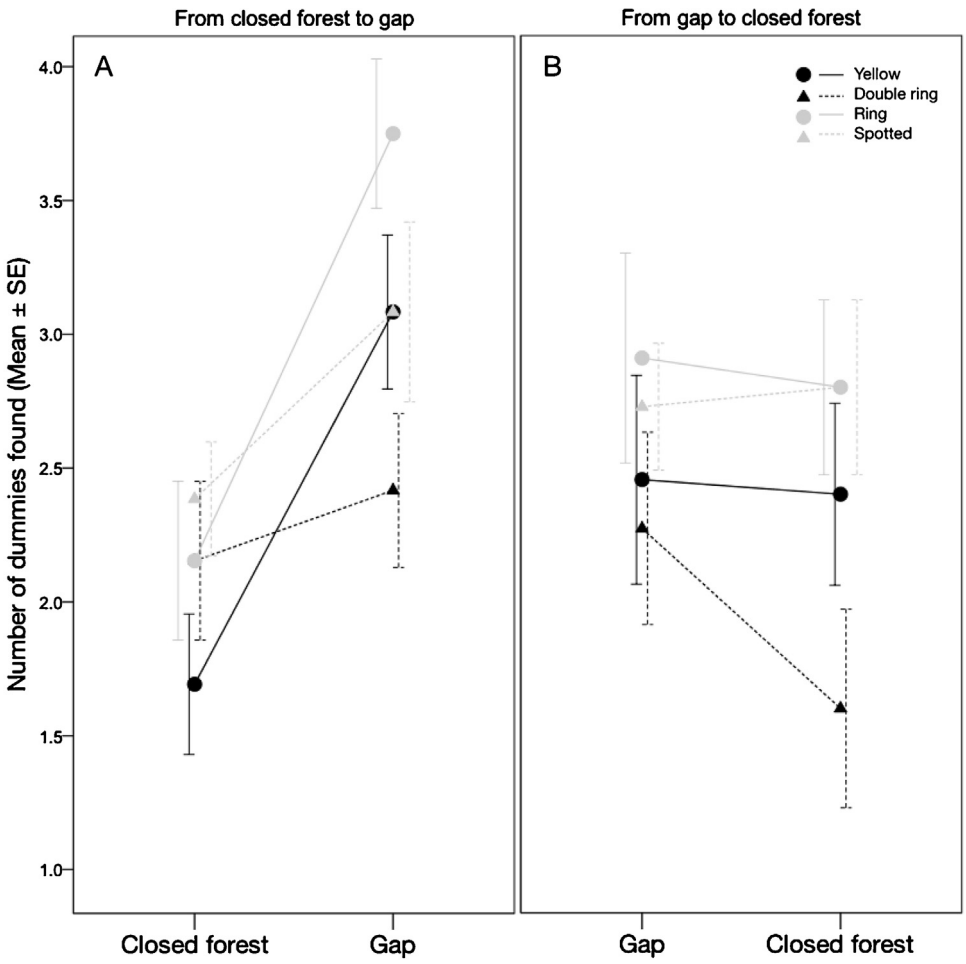

Fig. 4. Mean (±standard error) number of models of each morph found in gap and closed forest by human predators, going from closed forest to gap (A) and from gap to closed forest (B).

that some morphs seem to be more detectable in one of the light environments than in the other, but this detectability depends on whether the human predator went from closed forest to gap, or vice versa (Fig. 4). According to these analyses (Table 2), it seems that when going from closed canopy to gap, all morphs increase their detectability, except the double ring morph (i.e., complex), which appears to be equally difficult to detect in both light environments (Fig. 4). However, when human predators went from gap to closed forest, all morphs seemed to remain equally detectable except for the double ring morph, whose detectability decreased (Fig. 4).

4. Discussion

Our results suggest that differential detectability in altered light environments can be a key for understanding how several aposematic morphs can co-occur. Previous studies found that frogs with simpler colour patterns tend to arrive faster to novel environments (forest gaps) compared to individuals with more complex patterns. We did not find any evidence of an advantage for individuals with simple aposematic patterns in terms of higher survival in novel environments. However, individuals with complex colour patterns seemed to be less detectable. Altogether, the differential detectability among morphs could aid in the maintenance of colour-pattern polymorphism.

It has been suggested recently that aposematic species may have advantages over cryptic ones during the exploitation of newly available resources (Speed et al., 2010). That is because aposematic colouration grants individuals the protection to freely move around and explore, for example, new habitats as local predators would quickly learn that they are unpalatable (Speed et al., 2010). If prey can survive from the attack (Skelhorn and Rowe, 2006), or predators are neophobic (i.e., refuse to attack novel prey (Marples and Kelly, 1999)), or they have inherited tendencies to avoid such prey (Lindström et al., 1999a), these can indeed enhance the invasion success. Our results confirm this hypothesis because cryptic, brown frogs suffered significantly more attacks than aposematic frogs. Because frogs do not exist naturally in this area, we can assume that for most predators these frogs were novel prey. Thus, our experiment simulated a situation where novel prey invade a new environment.

There is evidence from previous studies that individuals of dyeing poison frogs with simpler colour patterns arrive first at newly formed treefall gaps (Rojas and Endler, unpublished data). If simplicity made aposematic signals easier to learn and remember, as detection theory suggests (Macmillan and Creelman, 1991), it would be reasonable to expect that, within a polymorphic aposematic species, individuals with more efficient colour patterns (better protected), could afford to invade new environments sooner than individuals bearing patterns that are less efficient at training predators. Alternatively, the aggregation of these frogs in canopy gaps might enhance predator learning and aid in the generalisation process among different signals, not only because of the known benefits that gregariousness confers to aposematic prey (Riipi et al., 2001; Gamberale and Tullberg, 1996; Hatle and Salazar, 2001; Alatalo and Mappes, 1996; Mappes and Alatalo, 1997), but

also because of increased encounters between predators and prey (Endler and Rojas, 2009).

Contrary to our expectations, we found no differences in attack rates between both aposematic morphs (simple vs. complex), i.e. neither morph was better protected from wild predators than the other. However, according to our second experiment, some of the morphs are less detectable by human predators than others. Especially hard to detect was the double ring morph, used in Experiment 1 as 'complex'. Therefore, there is a possibility that although the more visible (and potentially more efficient) simple pattern is easier to detect, it is also easier to learn and remember by naïve predators. More complex (and less detectable) signal, although less efficient in educating the predators, could benefit due to their lower detectability, particularly in a situation where a predator is either naïve or specialist, and ready to kill the defended prey (Valkonen et al., 2012). The consequence of this scenario is that there would be no differences in fitness between the morphs, as we observed in the field. This balance between lower detection and better efficiency would therefore allow for the co-existence of different morphs. Testing whether there are differences in signal efficacy, i.e. innate wariness and/or avoidance learning efficiency by predators towards simple and complex patterns, is the task of future work.

Overall, in our second experiment, more frog models were found in the gap that in the closed forest. This is not surprising considering that light environment influences how signals are perceived. The light environment of canopy gaps favours signal displays with yellow, and those have increased contrast if combined with blue (Endler, 1993; Théry and Endler, 2001). Those two colours are prevalent in *Dendrobates tinctorius* at the study site (Rojas and Endler, 2013). Interestingly, we found that the effect of light environment on the number of models found interacts with that of order. In other words, the number of wax models found by human predators depended not only of whether they were first in a gap or in a closed forest. It also depended on whether the human predators were first exposed to the models in a gap, and then in the closed forest, or vice versa.

If we assume that detectability correlates with signal efficiency (Lindström et al., 1999a,b), our results indicate that signal efficiency can change according to the light environment. In the frogs' natural environment gaps and closed forest form a dynamic mosaic, which can potentially allow the co-existence of multiple morphs. This means that in polymorphic, aposematic prey, the interaction of predator vision, environment and intrinsic characteristics of the colour patterns may favour some morphs under the same conditions that other forms would be selected against, or at least less favoured.

When we compare the results of our experiment with wild predators, with those obtained in the experiment with human predators within the gap treatment, we found that the trends are the same: no difference in the survival of the simpler (yellow) vs. the more complex (double ring). This is a very interesting result because (1) it shows that two visual predators behave the same towards different aposematic signals within the gap habitat; and (2) it reinforces the importance of the effect of light environment in the survival of individuals with different colour patterns. It could be argued that humans are not able to provide reliable information on prey detection in comparison to actual visual predators such as birds. However, some studies have shown that the perceptual abilities of birds and humans are similar under laboratory conditions, especially when the stimuli have been presented in uniform backgrounds (Penney et al., 2012; Beatty et al., 2005), and that human detection of different colour patterns reflects their relative survival in the wild (Karpestam et al., 2013). Considering that our experiment was done in the natural environment of the frogs (i.e. with their natural background and under natural light conditions), we believe that our findings provide novel and reliable insights on the importance of the joint role of predator experience and light environment in the maintenance of within-population variation in aposematic colour patterns.

In sum, it seems that simpler aposematic colour patterns do not have an advantage per se over complex patterns, but that the coexistence of variable aposematic signals within the same population may be mediated by differences in detectability. The ultimate consequence of this is that survival difference among morphs may vary among environments because differential light conditions can switch the order of magnitude of the costs and benefits of a signal from positive to negative. This supports the idea that aposematism and crypsis are not necessarily mutually exclusive strategies (Stevens, 2007; Mappes et al., 2005; Tullberg et al., 2005), but rather a continuum even within species. Our study shows how the light environment influences prey detectability and thus highlights the importance of taking light conditions into account in future studies on predation in the wild.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.beproc.2014.08.014.

References

Alatalo, R.V., Mappes, J., 1996. Tracking the evolution of warning signals. Nature 382, 708–710.

Allen, J.A., Clarke, B., 1968. Evidence for apostatic selection by wild passerines. Nature 220, 501–502.

Alvarado, J.B., Alvarez, A., Saporito, R.A., 2013. *Oophaga pumilio* (Strawberry Poison Frog), Baryphthengus martii (Rufous Motmot). Predator prey interactions. Herpetol. Rev. 44, 298.

Amézquita, A., Castro, L., Arias, M., González, M., Esquivel, C., 2013. Field but not lab paradigms support generalisation by predators of aposematic polymorphic prey: the *Oophaga histrionica* complex. Evolut. Ecol. 27, 769–782.

Beatty, C.D., Bain, R.S., Sherratt, T.N., 2005. The evolution of aggregation in profitable and unprofitable prey. Animal Behav. 70, 199–208.

Bohlin, T., Gamberale-Stille, G., Merilaita, S., ExnerovÁ, A., ŠTys, P., Tullberg, B.S., 2012. The detectability of the colour pattern in the aposematic firebug, Pyrrhocoris apterus: an image-based experiment with human 'predators'. Biol. J. Linnean Soc. 105, 806–816.

Bond, A.B., Kamil, A.C., 1998. Apostatic selection by blue jays produces balanced polymorphism in virtual prey. Nature 395, 594–596.

Bond, A.B., Kamil, A.C., 2002. Visual predators select for crypticity and polymorphism in virtual prey. Nature 415, 609–613.

Bond, A.B., Kamil, A.C., 2006. Spatial heterogeneity, predator cognition, and the evolution of color polymorphism in virtual prey. Proc. Natl. Acad. Sci. U.S.A. 103, 3214–3219.

Born, M., Bongers, F., Poelman, E.H., Sterck, F.J., 2010. Dry-season retreat and dietary shift of the dart-poison frog *Dendrobates tinctorius* (Anura: Dendrobatidae). Phyllomedusa 9, 37–52.

Bowers, D.M., 1993. Aposematic caterpillars: life-styles of the warningly colored and unpalatable. In: Stamp, N.E., Casey, T.M. (Eds.), Caterpillars: Ecological and Evolutionary Constraints on Foraging. Chapman and Hall, New York.
Brakefield, P.M., 1985. Polymorphic Mullerian mimicry and interactions with thermal melanism in ladybirds and a soldier beetle. A hypothesis. Biol. J. Linnean Soc. 26, 243–267.
Brodie, E.D., 1993. Differential avoidance of coral snake banded patterns by free-ranging avian predators in Costa Rica. Evolution 47, 227–235.
Brusa, O., Bellati, A., Meuche, I., Mundy, N.I., Prohl, H., 2013. Divergent evolution in the polymorphic granular poison-dart frog, *Oophaga granulifera*: genetics, coloration, advertisement calls and morphology. J. Biogeogr. 40, 394–408.
Chai, P., 1986. Field observations and feeding experiments on the responses of Rufous-tailed jacamars (*Galbula ruficauda*) to free-flying butterflies in a tropical rainforest. Biol. J. Linnean Soc. 29, 161–189.
Chai, P., 1988. Wing coloration of free-flying Neotropical butterflies as a signal learned by a specialized avian predator. Biotropica 20, 20–30.
Comeault, A.A., Noonan, B.P., 2011. Spatial variation in the fitness of divergent aposematic phenotypes of the poison frog, *Dendrobates tinctorius*. J. Evol. Biol. 24, 1374–1379.
Courtois, E.A., Pineau, K., Villette, B., Schmeller, D.S., Gaucher, P., 2012. Population estimates of *Dendrobates tinctorius* (Anura: Dendrobatidae) at three sites in French Guiana and first record of chytrid infection. Phyllomedusa 11, 63–70.
Crothers, L.R., Cummings, M.E., 2013. Warning signal brightness variation: sexual selection may work under the radar of natural selection in populations of a polytypic poison frog. Am. Nat. 181, E116–E124.
Cummings, M.E., Crothers, L.R., 2013. Interacting selection diversifies warning signals in a polytypic frog: an examination with the strawberry poison frog. Evol. Ecol. 27, 693–710.
Daly, J.W., Myers, C.W., Whittaker, N., 1987. Further classification of skin alkaloids from neotropical poison frogs (Dendrobatidae), with a general survey of toxic/noxious substances in the Amphibia. Toxicon 25, 1023–1095.
Darst, C.R., Cummings, M.E., 2006. Predator learning favours mimicry of a less-toxic model in poison frogs. Nature 440, 208–211.
Devillechabrolle, J., 2011. Mise en place et analyse d'un protocole pour le suivi à long terme d'amphibiens en forêt tropicale humide de Guyane Française. University of Marseille, Marseille, France, Masters Thesis.
Endler, J.A., 1986. Defense against predators. In: Feder, M.E.L.G.V. (Ed.), Predator Prey Relationships. Perspectives and Approaches from the Study of Lower Vertebrates.
Endler, J.A., 1988. Frequency-dependent predation, crypsis and aposematic coloration. Philos. Trans. R. Soc. London Ser. B-Biol. Sci. 319, 505–523.
Endler, J.A., 1991. Variation in the appearance of guppy color patterns to guppies and their predators under different visual conditions. Vision Res. 31, 587–608.
Endler, J.A., 1993. The color of light in forests and its implications. Ecol. Monogr. 63, 1–27.
Endler, J.A., 2012. A framework for analysing colour pattern geometry: adjacent colours. Biol. J. Linnean Soc. 107, 233–253.
Endler, J.A., Mappes, J., 2004. Predator mixes and the conspicuousness of aposematic signals. Am. Nat. 163, 532–547.
Endler, J.A., Rojas, B., 2009. The spatial pattern of natural selection when selection depends on experience. Am. Nat. 173, E62–E78.
Endler, J.A., Théry, M., 1996. Interacting effects of lek placement, display behavior, ambient light, and color patterns in three neotropical forest-dwelling birds. Am. Nat. 148, 421–452.
Exnerová, A., Štys, P., Fučíková, E., Veselá, S., Svádová, K., Prokopovvá, M., Jarošík, V., Fuchs, R., Landová, E., 2007. Avoidance of aposematic prey in European tits (Paridae): learned or innate? Behav. Ecol. 18, 148–156.
Exnerová, A., Svádová, K., Stys, P., Barcalová, S., Landová, E., Prokopovvá, M., Fuchs, R., Socha, R., 2006. Importance of colour in the reaction of passerine predators to aposematic prey: experiments with mutants of *Pyrrhocoris apterus* (Heteroptera). Biol. J. Linnean Soc. 88, 143–153.
Ford, E.B., 1945. Polymorphism. Biol. Rev. Cambridge Philosoph. Soc. 20, 73–88.
Forsman, A., Appelqvist, S., 1998. Visual predators impose correlational selection on prey color pattern and behavior. Behav. Ecol. 9, 409–413.
Fraser, S., Callahan, A., Klassen, D., Sherratt, T.N., 2007. Empirical tests of the role of disruptive coloration in reducing detectability. Proc. R. Soc. B-Biol. Sci. 274, 1325–1331.
Gamberale, G., Tullberg, B.S., 1996. Evidence for a more effective signal in aggregated aposematic prey. Anim. Behav. 52, 597–601.
Gamberale-Stille, G., Tullberg, B.S., 1999. Experienced chicks show biased avoidance of stronger signals: an experiment with natural colour variation in live aposematic prey. Evol. Ecol. 13, 579–589.
Hagman, M., Forsman, A., 2003. Correlated evolution of conspicuous coloration and body size in poison frogs (Dendrobatidae). Evolution, 57.
Ham, A.D., Ihalainen, E., Lindstrom, L., Mappes, J., 2006. Does colour matter? The importance of colour in avoidance learning, memorability and generalisation. Behav. Ecol. Sociobiol. 60, 482–491.
Hatle, J.D., Salazar, B.A., 2001. Aposematic coloration of gregarious insects can delay predation by an ambush predator. Environ. Entom. 30, 51–54.
Hegna, R.H., Saporito, R.A., Gerow, K.G., Donnelly, M.A., 2011. Contrasting colors of an aposematic poison frog do not affect predation. Ann. Zool. Fenn. 48, 29–38.
Heindl, M., Winkler, H., 2003. Interacting effects of ambient light and plumage color patterns in displaying Wire-tailed Manakins (Aves, Pipridae). Behav. Ecol. Sociobiol. 53, 153–162.
Ihalainen, E., Lindstrom, L., Mappes, J., Puolakkainen, S., 2008. Butterfly effects in mimicry? Combining signal and taste can twist the relationship of Mullerian co-mimics. Behav. Ecol. Sociobiol. 62, 1267–1276.
Joron, M., Mallet, J., 1998. Diversity in mimicry: paradox or paradigm? Trends Ecol. Evol. 13, 461–463.
Karpestam, E., Merilaita, S., Forsman, A., 2012. Reduced predation risk for melanistic pygmy grasshoppers in post-fire environments. Ecol. Evol. 2, 2204–2212.
Karpestam, E., Merilaita, S., Forsman, A., 2013. Detection experiments with humans implicate visual predation as a driver of colour polymorphism dynamics in pygmy grasshoppers. BMC Ecol. 13.
Langham, G.M., 2004. Specialized avian predators repeatedly attack novel color morphs of *Heliconius* butterflies. Evolution 58, 2783–2787.
Lindström, L., Alatalo, R.V., Lyytinen, A., Mappes, J., 2001. Strong antiapostatic selection against novel rare aposematic prey. Proc. Natl. Acad. Sci. U. S. A. 98, 9181–9184.
Lindström, L., Alatalo, R.V., Mappes, J., 1999a. Reactions of hand-reared and wild-caught predators toward warningly colored, gregarious, and conspicuous prey. Behav. Ecol. 10, 317–322.
Lindström, L., Alatalo, R.V., Mappes, J., Riipi, M., Vertainen, L., 1999b. Can aposematic signals evolve by gradual change? Nature 397, 249–251.
Lindström, L., Lyytinen, A., Mappes, J., Ojala, K., 2006. Relative importance of taste and visual appearance for predator education in Mullerian mimicry. Anim. Behav. 72, 323–333.
Maan, M.E., Cummings, M.E., 2009. Sexual dimorphism and directional sexual selection on aposematic signals in a poison frog. Proc. Natl. Acad. Sci. U. S. A. 106, 19072–19077.
Maan, M.E., Cummings, M.E., 2012. Poison frog colors are honest signals of toxicity, particularly for bird predators. Am. Nat. 179, E1–E14.
Macmillan, N.A., Creelman, C.D., 1991. Detection Theory: A User's Guide. Cambridge University Press, Cambridge.
Mallet, J., Joron, M., 1999. Evolution of diversity in warning color and mimicry: polymorphisms, shifting balance, and speciation. Ann. Rev. Ecol. Syst. 30, 201–233.
Mappes, J., Alatalo, R.V., 1997. Effects of novelty and gregariousness in survival of aposematic prey. Behav. Ecol. 8, 174–177.
Mappes, J., Marples, N., Endler, J.A., 2005. The complex business of survival by aposematism. Trends Ecol. Evol. 20, 598–603.
Marples, N.M., Kelly, D.J., 1999. Neophobia and dietary conservatism: two distinct processes? Evol. Ecol. 13, 641–653.
Marples, N.M., Vanveelen, W., Brakefield, P.M., 1994. The relative importance of color taste and smell in the protection of an aposematic insect *Coccinella septempunctata*. Anim. Behav. 48, 967–974.
Master, T.L., 1999. Predation by Rufous Motmot on black-and-green poison dart frog. Wilson Bull. 111, 439–440.
McGuire, L., Van Gossum, H., Beirinckx, K., Sherratt, T.N., 2006. An empirical test of signal detection theory as it applies to Batesian mimicry. Behav. Proc. 73, 299–307.
Medina, I., Wang, I.J., Salazar, C., Amézquita, A., 2013. Hybridization promotes color polymorphism in the aposematic harlequin poison frog Oophaga histrionica. Ecol. Evol.
Myers, C.W., Daly, J.W., 1976. Preliminary evaluation of skin toxins and vocalizations in taxonomic and evolutionary studies of poison-dart frogs (Dendrobatidae). Bull. Amer. Mus. Nat. Hist. 157, 177–262.
Myers, C.W., Daly, J.W., 1983. Dart-poison frogs. Sci. Am. 248, 96–105.
Nokelainen, O., Hegna, R.H., Reudler, J.H., Lindstedt, C., Mappes, J., 2012. Trade-off between warning signal efficacy and mating success in the wood tiger moth. Proc. R. Soc. B-Biol. Sci. 279, 257–265.
Nokelainen, O., Lindstedt, C., Mappes, J., 2013. Environment-mediated morph-linked immune and life-history responses in the aposematic wood tiger moth. J. Anim. Ecol. 82, 653–662.
Nokelainen, O., Valkonen, J., Lindstedt, C., Mappes, J., 2014. Changes in predator community structure shifts the efficacy of two warning signals in Arctiid moths. J. Anim. Ecol. 83, 598–605.
Noonan, B.P., Comeault, A.A., 2009. The role of predator selection on polymorphic aposematic poison frogs. Biol. Lett. 5, 51–54.
Noonan, B.P., Gaucher, P., 2006. Refugial isolation and secondary contact in the dyeing poison frog *Dendrobates tinctorius*. Mol. Ecol. 15, 4425–4435.
O'Donald, P., Majerus, M.E.N., 1984. Polymorphism of melanic ladybirds maintained by frequency-dependent sexual selection. Biol. J. Linnean Soc. 23, 101–111.
Paluh, D.J., Hantak, M.M., Saporito, R.A., 2014. A test of aposematism in the dendrobatid poison frog *Oophaga pumilio*: the importance of movement in clay model experiments. J. Herpetol. 48, 249–254.
Penney, H.D., Hassall, C., Skevington, J.H., Abbott, K.R., Sherratt, T.N., 2012. A comparative analysis of the evolution of imperfect mimicry. Nature 483, 461–464.
Pfennig, D.W., Harper Jr., G.R., Brumo, A.F., Harcombe, W.R., Pfennig, K.S., 2007. Population differences in predation on Batesian mimics in allopatry with their model: selection against mimics is strongest when they are common. Behav. Ecol. Sociobiol. 61, 505–511.
Richards-Zawacki, C.L., Yeager, J., Bart, H.P.S., 2013. No evidence for differential survival or predation between sympatric color morphs of an aposematic poison frog. Evol. Ecol. 27, 783–795.
Riipi, M., Alatalo, R.V., Lindstrom, L., Mappes, J., 2001. Multiple benefits of gregariousness cover detectability costs in aposematic aggregations. Nature 413, 512–514.
Rojas, B., 2012. The Apparent Paradox of Colour Variation in Aposematic Poison Frogs. School of Life and Environmental Sciences, Deakin University, Geelong, Australia, PhD Dissertation.

Rojas, B., Devillechabrolle, J., Endler, J.A., 2014. Paradox lost: variable colour-pattern geometry is associated with differences in movement in aposematic frogs. Biol. Lett. 10, 20140193.
Rojas, B., Endler, J.A., 2013. Sexual dimorphism and intra-populational colour pattern variation in the aposematic frog *Dendrobates tinctorius*. Evol. Ecol. 27, 739–753.
Rowland, H.M., Ihalainen, E., Lindström, L., Mappes, J., Speed, M.P., 2007. Co-mimics have a mutualistic relationship despite unequal defences. Nature 448, 64–67.
Saporito, R.A., Zuercher, R., Roberts, M., Gerow, K.G., Donnelly, M.A., 2007. Experimental evidence for aposematism in the dendrobatid poison frog Oophaga pumilio. Copeia, 1006–1011.
Sherratt, T.N., Rashed, A., Beatty, C.D., 2004. The evolution of locomotory behavior in profitable and unprofitable simulated prey. Oeco 138, 143–150.
Skelhorn, J., Rowe, C., 2006. Avian predators taste-reject aposematic prey on the basis of their chemical defence. Biol. Lett. 2, 348–350.
Speed, M.P., Brockhurst, M.A., Ruxton, G.D., 2010. The dual benefits of aposematism: predator avoidance and enhanced resource collection. Evolution 64, 1622–1633.
Stevens, M., 2007. Predator perception and the interrelation between different forms of protective coloration. Proc. R. Soc. B-Biol. Sci. 274, 1457–1464.
Stevens, M., Yule, D.H., Ruxton, G.D., 2008. Dazzle coloration and prey movement. Proc. R. Soc. B-Biol. Sci. 275, 2639–2643.
Summers, K., Clough, M.E., 2001. The evolution of coloration and toxicity in the poison frog family (Dendrobatidae). Proc. Natl. Acad. Sci. U. S. A. 98, 6227–6232.
Svádová, K., Exnerová, A., Stys, P., Landová, E., Valenta, J., Fucíková, A., Socha, R., 2009. Role of different colours of aposematic insects in learning, memory and generalization of naive bird predators. Anim. Behav. 77, 327–336.
Théry, M., Endler, J.A., 2001. Habitat selection ambient light and colour patterns in some lek-displaying birds. In: Bongers, F., Charles-Dominique, P., Forget, P.-M., Théry, M. (Eds.), Nouragues: Dynamics and Plant-animal Interactions in a Neotropical Rainforest. Kluwer Academic Publisher, Dordrecht, The Netherlands.
Thomas, R.J., Bartlett, L.A., Marples, N.M., Kelly, D.J., Cuthill, I.C., 2004. Prey selection by wild birds can allow novel and conspicuous colour morphs to spread in prey populations. Oikos 106, 285–294.
Tullberg, B.S., Merilaita, S., Wiklund, C., 2005. Aposematism and crypsis combined as a result of distance dependence: functional versatility of the colour pattern in the swallowtail butterfly larva. Proc. R. Soc. B-Biol. Sci. 272, 1315–1321.
Ueno, H., Sato, Y., Tsuchida, K., 1998. Colour-associated mating success in a polymorphic Ladybird Beetle, *Harmonia axyridis*. Funct. Ecol. 12, 757–761.
Valkonen, J.K., Nokelainen, O., Jokimäki, M., Kuusinen, E., Paloranta, M., Peura, M., Mappes, J., 2014. From deception to frankness: benefits of ontogenetic shift in the anti-predator strategy of alder moth Acronicta alni larvae. Curr. Zool. 60, 114–122.
Valkonen, J.K., Nokelainen, O., Niskanen, M., Kilpimaa, J., Bjorklund, M., Mappes, J., 2012. Variation in predator species abundance can cause variable selection pressure on warning signaling prey. Ecol. Evol. 2, 1971–1976.
Wollenberg, K.C., Lotters, S., Mora-Ferrer, C., Veith, M., 2008. Disentangling composite colour patterns in a poison frog species. Biol. J. Linnean Soc. 93, 433–444.